January 6, 1957

My dear Pardee:

I was very glad to finally have a chance to meet you, and begin to discuss some of the problems that interest both of us. We had a very pleasant time at Berkeley, largely on account of our many friends there, and hope we can renew that experience often.

As to penicillin, and your ms: let me say that I don't think we can still be quite sure of the primary effect; the enclosed note, however, gives my argument that the principal lethal effect can be circumvented by osmotic protection-- is this a sufficient criterion to distinguish 'wall' from 'membrane'? I have thought the simplest hypothesis to work to test was that the target was the wall-polymerase itself, but even at that this could be the membrane you're talking about! Mainly I would urge that for you to publish at this particular point might lead to some confusion, and I hope you will consider trying to extend your experiments to sucrose-Mg-protected protoplasts, which you should be able to do, and which might be quite decisive, before coming out with this pre-climactic account. But of course this is entirely a matter for your own judgment. (If you do send this to J. Bact., would you mention to Porter that I had already seen the ms. and discussed it with you, and for that reason would prefer not to have it come to me as a reviewer-- this will save some time.) Naturally, I have the hope that when you do reproduce the protoplast results you may change some of your own viewpoints in details. (I have to add that we have been finding in <u>some</u> runs that sucrose-protected protoplasts are not entirely stable, and may lose much of their initial viability--that is, revertibility-- on continued incubation in the penicillin-sucrose-Mg-broth.)

You did ask me to comment on the ms., so here goes. Do what you want with these notes:

p.1 M&M. Is it of any possible significance that the various mutants come from a variety of strains, not all B?

p.3 I think that J.Bact. would ~~per~~ prefer Chloramphenicol to Chloromycetin which is the tradename.

p.7 lines 1-2. Isn't this a reference to my 1950 paper in J Bact?

p.9; I mentioned to you that the citrate spoils Davis' medium for making protoplasts. In a similar medium without citrate, but with penicillin, Mg + sucrose, swellings become quite noticeable after about 1 hour; it takes about 4 to get the full ballooning, since this needs an appreciable mass increment.

p.14 back to the old question, which is primary?

p. 19 here of course, I have to disagree. What makes you ~~see~~ say the peni-

cillin protoplasts
don't resemble those from lysozyme? If you mean it, you should specify how. Now, I think you may be referring to van Niel's class experiment at P.G.-- however, he had run the treatments xxxxx under rather unfavorable conditions, and for too short a time at room temperature, and therefore caught only the early stages. We started a xxxxxxx repetition together, and got somewhat further. Of course, these protoplasts are larger, since the final shedding of the old wall requires a considerable increase (by growth) of the protoplast: it also continues to grow (only in mass so long as kept in broth) on further incubation.

As to the rate considerations, the microscopic observations favor a special sensitivity of the 'dividing pole' of the wall, perhaps because this is the portion whihh grows most actively. You might then get some local attenuation fairly rapidly, but it would take a substantial growth of the protoplast before it bulged out.

I couldn't understand Mitchell & Moyle's paper, no less than their result. But I would expect to find protoplast formation under a variety of conditions where there was discrepant growth of mass and wall.

With best wishes for the new year,

Yours sincerely,

Joshua Lederberg